Jones, P., Prudhoe, K., and Robson, T. (1973b), *Biochem. J. 120,* 361.
Jones, P., Robson, T., and Brown, S. B. (1973a), *Biochem. J. 120,* 353.
Kremer, M. L. (1965a), *Nature (London) 205,* 384.
Kremer, M. L. (1965b), *Trans. Faraday Soc. 61,* 1453.
Portsmouth, D., and Beal, E. A. (1971), *Eur. J. Biochem. 19,* 479.
Prudhoe, K. (1971), Ph.D. Thesis, The University, Newcastle-upon-Tyne.
Robson, T. (1973), Ph.D. Thesis, The University, Newcastle-upon-Tyne.
von Euler, H., and Josephson, K. (1927), *Justus Liebigs Ann. Chem. 456,* 111.

Characterization of the Surface Protein Components in Adipocyte Plasma Membranes†

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ABSTRACT: Plasma membranes isolated from rat adipocytes were examined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Gels stained with Coomassie Blue indicated the presence of more than 20 protein components with an apparent molecular weight range of 178,000–15,000. Staining with Schiff reagent further indicated the presence of two major glycoproteins of molecular weight 100,000 and 81,000 and several minor components. The lactoperoxidase-catalyzed iodination technique was used to determine which membrane components were located on the surface of the intact cell and to monitor changes in exposure of surface components in the presence of hormones and concanavalin A. Analysis of polyacrylamide gels of the membranes isolated from labeled cells indicated that four membrane components were labeled and that two of these components corresponded in electrophoretic mobility to the major glycoprotein bands. The pattern of incorporation of iodine was shown to depend on the nature of the buffer in which the adipocytes were suspended. Iodination was also carried out on the particulate fraction derived from homogenized cells, on the isolated plasma membrane, and on the lipid extracted plasma membrane. The analysis of the labeling patterns suggested that several membrane components were buried and only became available for iodination upon membrane perturbation. Radioactive label was also shown to be associated with a low molecular weight membrane component which could possibly be lipid or glycolipid. Iodination of intact adipocytes appeared to have no effect on the biological integrity of these cells as assayed by the conversion of glucose to carbon dioxide. Incubation of isolated adipocytes with glucagon, insulin, or concanavalin A had significant effects on the quantitative distribution of radioactivity incorporated into the membrane proteins.

The relationship of cell surface topology to the functional properties of animal cells has been extensively investigated in many systems (Mehrishi, 1972). A necessary aspect of this research is the characterization of these complex assemblies. The spatial arrangement of the components of surface membranes has been studied using a variety of chemical and enzymatic procedures (Berg, 1969; Steck *et al.*, 1971; Bretscher, 1971a; Rifkin *et al.*, 1972). The lactoperoxidase-catalyzed iodination of tyrosine in various proteins (Marchalonis, 1969) has been used to selectively label the protein components on the external surface of plasma membranes in various cell systems. The technique has been successfully applied to erythrocytes (Phillips and Morrison, 1970, 1971a,b; Hubbard and Cohn, 1972), lymphocytes (Marchalonis *et al.*, 1971), platelets (Phillips, 1972; Nachman *et al.*, 1973), mouse fibroblasts (L cells) (Poduslo *et al.*, 1972), *Micrococcus lysodeikticus* (Salton *et al.*, 1972), and to microsomal (Kreibich *et al.*, 1974) and mitochondrial membranes (Huber and Morrison, 1973) from rat liver.

The regulation of adipose cell metabolism has been extensively examined (Jeanrenaud and Hepp, 1970; Avruch *et al.*, 1972). The plasma membrane obtained from these cells has been shown to be the site of action of several hormones and to contain the hormone responsive adenylate cyclase system (Cuatrecasas, 1969, 1972; Birnbaumer *et al.*, 1969; Birnbaumer and Rodbell, 1969; Laudat *et al.*, 1972).

A study of the surface topology of the adipocyte plasma membrane should contribute significantly to the understanding of its regulatory functions. This report presents the results of a characterization of the protein and glycoprotein components of these membranes. Lactoperoxidase-catalyzed iodinations of intact adipose cells and plasma membrane preparations have been used to reveal the disposition of components on the membrane surface. The effect of hormones and the plant lectin concanavalin A on the exposure of surface components has also been investigated. While this manuscript was in preparation, a paper by Czech and Lynn (1973b) appeared in which is reported the iodination of intact adipocytes and isolated plasma membranes. The relevant portions of our report generally support the major conclusions of the aforementioned paper.

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Materials and Methods

Collagenase, pepsin, and ribonuclease were obtained from Worthington Biochemical Corp. Bovine serum albumin, type V, was obtained from Nutritional Biochemicals Corp. Acrylamide and methylenebisacrylamide were purchased from Eastman Chem. Corp. Lactoperoxidase from milk, phosphorylase *a*, bovine γ-globulin, crystalline glucagon, sodium dodecyl sulfate (SDS),[1] Coomassie Brilliant Blue, Bromophenyl Blue, concanavalin A, type IV, and methyl α-D-mannoside were obtained from Sigma Chemical Co. Carrier-free ^{125}I and [U-^{14}C]glucose were obtained from New England Nuclear. Bovine zinc insulin (Lot No. 493-88GPD-017) was the product of Eli Lilly and Co.

Preparation of Adipocytes and Adipocyte Plasma Membranes. Adipocytes were isolated from the epididymal fat pads of Sprague-Dawley rats (180–230 g) using the procedures of Rodbell (1964, 1966). All procedures were carried out in plastic vessels. Prior to iodination the isolated cells were washed three times with phosphate-buffered saline (0.15 M NaCl–1.0 mM EDTA–0.05 M sodium phosphate (pH 7.4)) or with Krebs-Ringer bicarbonate buffer without bovine serum albumin. Plasma membranes were prepared from the iodinated or unmodified adipocytes according to the procedures of McKeel and Jarett (1970), as modified by Carter *et al.* (1972). Prior to fractionation the cells were washed three times with Tris-sucrose buffer (0.01 M Tris–0.25 M sucrose–1 mM EDTA (pH 7.4)). The plasma membranes were isolated from the membrane band on the sucrose gradient by dilution with 6–8 volumes of Tris-sucrose buffer and centrifugation for 30 min at 40,000*g*. When the 24,000*g* pellet was to be iodinated it was resuspended in phosphate-buffered saline. Following iodination, the material was washed twice with the Tris-sucrose buffer before isolation of the plasma membrane was resumed. The plasma membrane fraction was suspended in 0.1–0.2 ml of 0.01 Tris–1.0 mM EDTA (pH 7.4). SDS (10%) was added to give a final concentration of 1% (w/v). When the plasma membrane was to be iodinated SDS was omitted. Following iodination, the material was washed twice with the Tris-sucrose buffer before solubilization with SDS. The mitochondrial fraction from unlabeled cells was also removed from the linear sucrose gradient and recovered as described for plasma membranes.

Lipid Extraction Procedures. Lipids were extracted from the plasma membrane fraction according to the method of Bligh and Dyer (1959). The resulting proteinaceous precipitate was resuspended in phosphate-buffered saline by vigorous vortexing. Nitrogen was bubbled through the suspension to remove traces of organic solvent prior to the iodination. Lipid extractions were also performed on adipocyte homogenates which had been previously iodinated. In this case the residue from the chloroform–methanol extraction was also extracted with 1-butanol or 95% ethanol at room temperature to ensure complete delipidation (Lenard, 1970; Czech and Lynn, 1973a).

Iodination Procedures. Intact adipocytes, 24,000*g* pellets, plasma membranes, and lipid extracted plasma membranes were iodinated by a modification of the lactoperoxidase-catalyzed iodination procedure described by Marchalonis *et al.* (1971) and Phillips (1972). The material to be iodinated was suspended in phosphate-buffered saline or Krebs-Ringer bicarbonate buffer without bovine serum albumin. A typical reaction was carried out on 10^6 cells or corresponding membrane preparations in a volume of 2.0 ml. To this stirred suspension was added lactoperoxidase (10 μl of 2.5×10^{-5} M), $Na^{127}I$ (5 μl of 1×10^{-4} M), and carrier-free $Na^{125}I$ (30-200 μCi; specific activity 17 Ci/mg) per milliliter of reaction mixture. The reaction was initiated by the addition of H_2O_2 (10 μl of 1×10^{-3} M) every 30 sec for 5 min. Three volumes of buffer was then added, and the iodinated material was recovered and washed as described above. The appropriate control reaction, omitting lactoperoxidase, was also carried out. Iodinated whole cells were washed and recovered by flotation upon low speed centrifugation. Whole cells to be iodinated in the presence of hormone were first incubated for 10 min at room temperature with 250 μunits of insulin or 15 μg of glucagon/ml of suspension. Reagents for iodination were then added and the reaction was performed as described above. Adipocytes were also iodinated in phosphate-buffered saline in the presence of EDTA and 1% bovine serum albumin.

For iodination of intact adipocytes in the presence of concanavalin A, either 5 or 150 μg of the plant lectin was added per ml of cell suspension in Krebs-Ringer bicarbonate buffer. The mixture was stirred very slowly for 1 hr at room temperature before the cells were iodinated as described above. Following iodination the cells were centrifuged to remove unbound reactants and concanavalin A, and then incubated for 1 hr at 37° in Krebs-Ringer bicarbonate buffer containing 50 mM methyl α-D-mannoside to displace bound concanavalin A. After two more washes, the cells were processed to obtain plasma membranes as described above.

Gel Electrophoresis. The isolated plasma membrane fraction was solubilized in 1% SDS and 2% β-mercaptoethanol, and the resulting clear solution was heated at 55° for 45 min. Bromophenol Blue and sucrose were added, and 100-μl aliquots containing 50–100 μg of protein were electrophoresed on 7.5% gels (90 × 6 mm) containing 0.1% SDS and 0.5 M urea as previously described (Gurd *et al.*, 1972). Gels were stained for protein with Coomassie Brilliant Blue according to Fairbanks *et al.* (1971), omitting the second stepdown stain, and for carbohydrate with the periodic acid–Schiff procedure (Fairbanks *et al.*, 1971). Gels were scanned at 610 nm for protein and 560 nm for carbohydrate on a Gilford 2400 spectrophotometer equipped with a linear transport assembly. The distribution of ^{125}I in the gel was ascertained by slicing the gel into 1-mm discs using a Misco 3015 gel slicer and counting the γ emissions from each slice on a Model 402 Packard Auto-Gamma spectrometer. The gel system was calibrated for molecular weights using γ-globulin, phosphorylase *a*, bovine serum albumin, pepsin, and ribonuclease as standards. The amount of radioactivity associated with lipid extracts was also determined.

Additional Procedures. Protein concentrations were determined by the method of Lowry *et al.* (1951), as modified by Hartree (1972), using bovine serum albumin as a standard. Hydrogen peroxide concentrations were estimated from the optical density at 230 nm using a molar extinction coefficient of 72.4. Adipocyte viability after iodination was estimated by the glucose oxidation method (Rodbell, 1964). Cells washed with phosphate-buffered saline as well as cells remaining in Krebs-Ringer bicarbonate–albumin buffer served as the appropriate controls.

Results

Protein and Glycoprotein Composition of Adipocyte

[1] Abbreviation used is: SDS, sodium dodecyl sulfate.

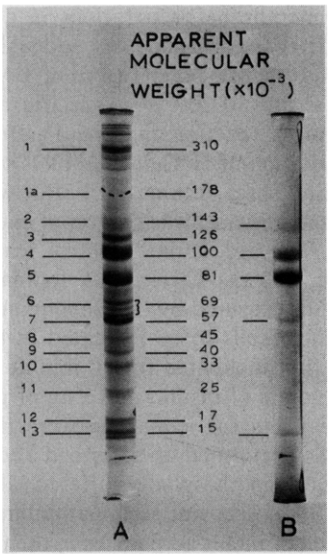

FIGURE 1: Polyacrylamide gel electrophoretic analysis of unlabeled adipocyte plasma membranes. Plasma membranes (approximately 100 μg of protein) were solubilized in 1% SDS and 2% β-mercaptoethanol and electrophoresed on 7.5% acrylamide gels as described in Materials and Methods. Duplicate gels were stained for protein with Coomassie Blue (gel A) and for carbohydrate with periodic acid–Schiff reagent (gel B). Apparent molecular weights were obtained as described in Materials and Methods. Alternate position of high molecular weight band (1a) is designated (- - -). Bands of lower mobility than band 1 appear to be artifactual.

Plasma Membranes. SDS–polyacrylamide gels of the plasma membranes derived from rat adipocytes displayed the characteristic patterns shown in Figure 1. Separate gels were stained for protein (Coomassie Blue) and for glycoprotein (Schiff reagent). More than 20 protein components with an apparent molecular weight range of 15,000–178,000 can be resolved by this procedure. The Schiff reagent stain indicated the presence of two major glycoproteins with molecular weights of 100,000 and 81,000, which coelectrophorese with the two most heavily stained protein bands (4 and 5). Three minor glycoprotein components with molecular weights of 143,000, 57,000, and 15,000 were also observed. Densitometer scans of these gels are shown in Figure 2. The mitochondrial band which was isolated from the linear sucrose gradient was also analyzed by SDS–polyacrylamide gel electrophoresis. The gel stained for protein with Coomassie Blue revealed more than 15 resolved bands with a molecular weight range of 18,000–180,000.

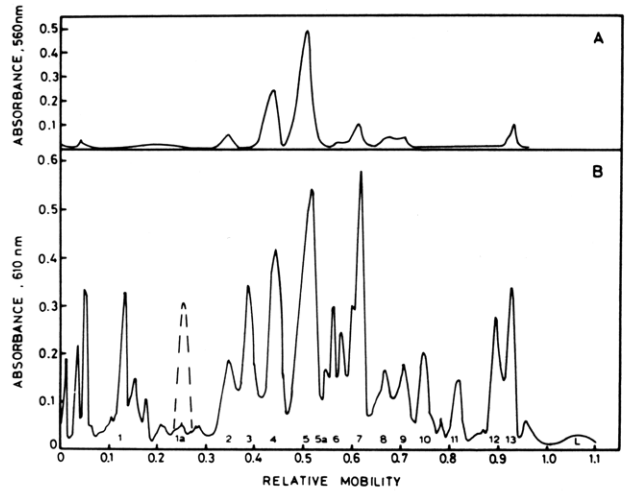

FIGURE 2: Densitometer scans of polyacrylamide gels of adipocyte plasma membranes: (A) periodic acid–Schiff stained gel containing 120 μg of membrane protein; (B) Coomassie Blue stained gel containing 120 μg of membrane protein.

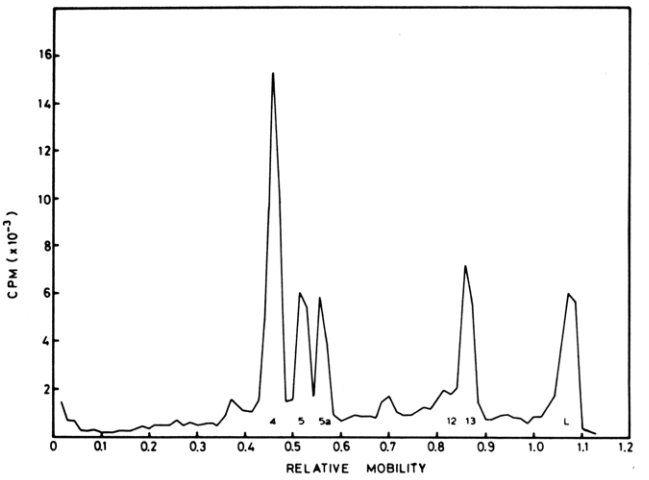

FIGURE 3: Distribution of ^{125}I in adipocyte plasma membranes. Intact adipocytes were iodinated with lactoperoxidase and sodium iodide (375 μCi) and the isolated membranes (200 μg) solubilized in SDS and electrophoresed as described in Materials and Methods. The labeled peaks are numbered to indicate the position of the corresponding protein band as determined by Coomassie Blue staining. L indicates the position of the low molecular weight membrane components.

Iodination of Intact Adipocytes. Adipocytes isolated from epididymal fat pads were iodinated utilizing the lactoperoxidase-catalyzed iodination system as previously described. Following the reaction, the plasma membrane was isolated by sucrose gradient centrifugation. Essentially all of the counts applied to the gradient were located in the plasma-membrane fraction. Approximately 0.1% of the iodine used was incorporated into this fraction. In the absence of lactoperoxidase less than 5% of the counts was taken up by the cells. These counts were shown to be dialyzable and thus not covalently bound to the cells. The distribution of the radioactive label on a polyacrylamide gel of plasma membranes isolated from iodinated intact adipocytes is shown in Figure 3. The majority of the incorporated label was found at positions corresponding to the protein bands 4, 5, 5a, 12, and 13. Two of these labeled protein peaks (4 and 5) correspond in electrophoretic mobility to the bands containing the two major glycoproteins. When iodination of intact adipocytes was carried out in the presence of 1% bovine serum albumin in phosphate-buffered saline containing EDTA, identical labeling patterns were obtained. The total incorporation of ^{125}I into the membrane proteins was reduced, however, as a result of a significant iodination of the added serum protein. When gels containing the labeled membranes were stained with Coomassie Blue no differences could be discerned between them and stained gels of unlabeled material. Radioactivity was also observed to run ahead of the tracking dye. Gels were observed to be slightly turbid in this region but did not stain with Coomassie Blue.

Adipocytes which had been iodinated retained full biological integrity as assayed by their ability to convert [U-^{14}C]glucose to $^{14}CO_2$. Cells exposed to the iodinating environment with or without lactoperoxidase showed no decrease in activity as compared to cells that were never removed from the Krebs-Ringer bicarbonate buffer–1% albumin.

Iodination of Homogenized Adipocytes and Lipid-Extracted Plasma Membranes. Significantly more radioactive

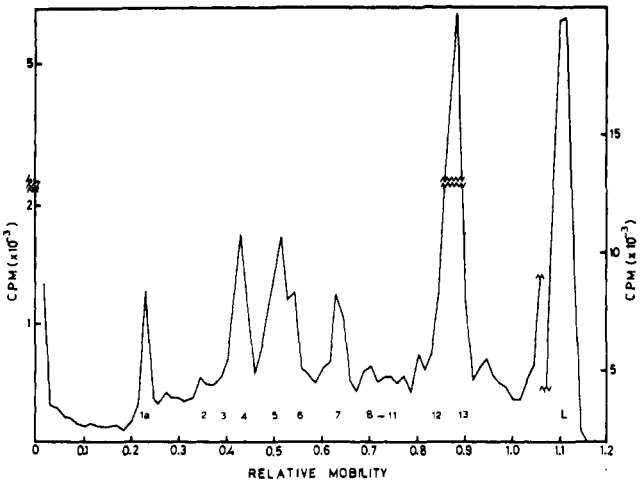

FIGURE 4: Distribution of ^{125}I in adipocyte plasma membranes. The pellet from homogenized adipocytes was iodinated with lactoperoxidase and sodium iodide (140 μCi) as described in Materials and Methods, and the isolated membranes (40 μg) were electrophoresed in the SDS-polyacrylamide gel system. The scale for peak L is located on the right side of the figure.

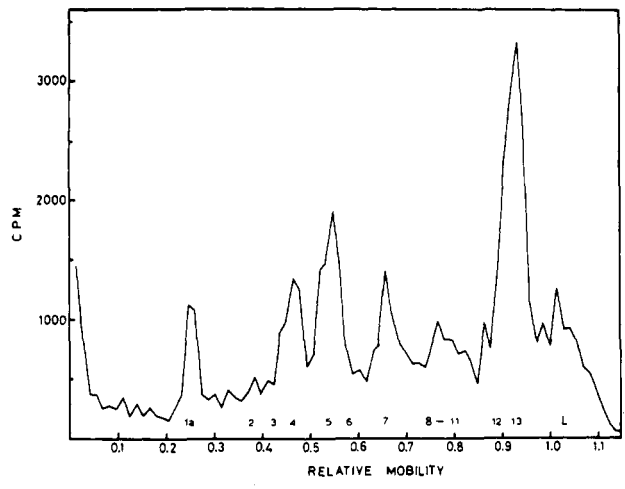

FIGURE 5: Distribution of ^{125}I in adipocyte plasma membrane proteins. Lipids were extracted from the isolated plasma membranes prior to iodination with lactoperoxidase and sodium iodide (36 μCi) as described in Materials and Methods. The labeled proteins (80 μg) were then electrophoresed in the SDS-polyacrylamide gel system.

label (~1% of iodine used) was incorporated into perturbed membrane systems than into membranes of the intact cell, which result is consistent with previously reported data (Poduslo *et al.*, 1972; Czech and Lynn, 1973b). In the present studies the stained acrylamide gels of the plasma membrane fractions isolated from iodinated homogenates and of lipid extracted membranes displayed the same protein pattern as that obtained from the membranes isolated from the intact cell. The analysis of the incorporated radioactive label, however, indicated a pattern altered from that derived from labeled intact cells. The pattern of ^{125}I incorporation into the adipocyte homogenate pellet is shown in Figure 4. The relative amount of label in peak 4 has been reduced while peaks 5 and 6 have been increased. The protein components in peaks 1a and 7–11, which were largely resistant to iodination in the intact cell, have now been modified, while material in peaks 12 and 13 has now become the most highly labeled protein in the gel. The low molecular weight material running ahead of the tracking dye has also become considerably more labeled.

When the isolated plasma membrane fraction was extracted using the Bligh and Dyer technique prior to iodination, the pattern of radioactivity on polyacrylamide gels, as shown in Figure 5, was similar to that obtained from the homogenate pellet, with the exception that considerably less radioactive material migrated ahead of the tracking dye. More vigorous extraction of iodinated plasma membranes resulted in complete removal of radioactivity from this position. SDS-polyacrylamide gel electrophoresis of the Bligh and Dyer extract afforded one band corresponding in position to the material running ahead of the tracking dye. The iodinated homogenate pellet was also extracted using the Bligh and Dyer technique. This procedure again resulted in the removal of all radioactivity running ahead of the tracking dye.

Effects of Buffer, Hormones, and Concanavalin A on Intact Adipocyte Iodination. Several experiments were performed to determine whether the radioactive labeling pattern described above was sensitive to alterations in the incubation medium, to the presence of hormones, or to concanavalin A. The labeling patterns on all polyacrylamide gels under the above conditions were qualitatively similar; however, a quantitative comparison of the glycoprotein region of the gels (Figures 6 and 7) suggested that the relative amount of radioactive label in each of the protein peaks (4, 5 and 5a) was sensitive to the particular incubation conditions. In all cases these peaks contained approximately 80% of the radioactivity incorporated into the plasma membrane proteins. The results shown in Figures 6 and 7 have been normalized so that data from the different experiments may be directly compared to best illustrate the observed differences in the labeling patterns. All experiments were repeated several times with a reproducibility of ±2% in the reported percentage values.

The labeling pattern obtained from iodinating adipocytes in Krebs-Ringer bicarbonate buffer (Figure 6a) was quantitatively different from the pattern obtained when adipocytes were iodinated in phosphate-buffered saline and EDTA (Figure 7a). The addition of 250 μunits of zinc insulin to intact adipocytes appeared to have a negligible effect on the incorporation of radioactive label into peaks 4, 5, and

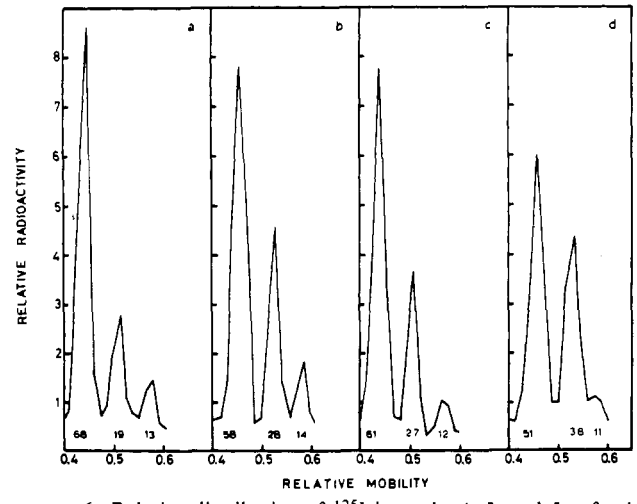

FIGURE 6: Relative distribution of ^{125}I in peaks 4, 5, and 5a of gels containing plasma membrane proteins from intact adipocytes iodinated in Krebs-Ringer bicarbonate buffer: (a) no additions, (b) 250 μunits of zinc insulin/ml of cell suspension, (c) 15 μg of glucagon/ml of cell suspension, (d) 250 μg of concanavalin A/ml of cell suspension. The number under each peak is the percentage of incorporated radioactive label.

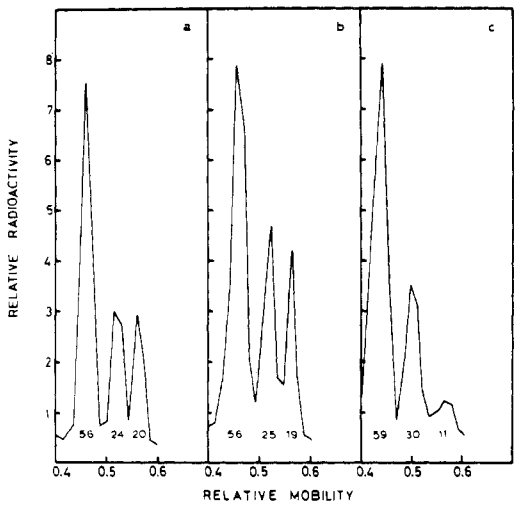

FIGURE 7: Relative distribution of ^{125}I in peaks 4, 5, and 5a of gels containing plasma membrane proteins from intact adipocytes iodinated in phosphate-buffered saline: (a) no additions, (b) 250 μunits of zinc insulin/ml of cell suspension, (c) 15 μg of glucagon/ml of cell suspension. The number under each peak is the percentage of incorporated radioactive label.

5a in phosphate-buffered saline with EDTA as seen in Figure 7a and b; however, in Krebs-Ringer bicarbonate buffer a significant alteration was observed (Figure 6a and b). The addition of glucagon (15 μg) resulted in a significant decrease in the amount of radioactivity associated with peak 5a in phosphate-buffered saline (Figure 7a and c). In Krebs-Ringer bicarbonate buffer a significant decrease (37%) in the ratio of the radioactivity in peaks 4 to 5 was observed as compared to the untreated cells (Figure 6a and c).

Adipocytes were also iodinated in the presence of both low (5 μg/ml) and high (250 μg/ml) concentrations of concanavalin A in Krebs-Ringer bicarbonate buffer. In the former case, the plant lectin had an effect similar to that produced by the addition of zinc insulin (Figure 6b). That the lectin itself is labeled during the experiment and not completely displaced by methyl α-D-mannoside is evident from the appearance in the gels of an additional radioactive band at a position corresponding to an apparent molecular weight of 22,000. Cunningham *et al.* (1972) have reported this molecular weight for the naturally occurring subunits of concanavalin A. The distribution of radioactivity in membranes of intact adipocytes iodinated in the presence of saturating amounts of the lectin, however, is distinctly different (Figure 6d), where the ratio of radioactivity in peak 4 to peak 5 has decreased by 62%.

Discussion

In this study, the hormone responsive plasma membrane from rat adipocytes has been characterized with respect to its protein and glycoprotein components using SDS–polyacrylamide gel electrophoresis. The protein components of the adipocyte mitochondrial fraction have also been characterized by the same procedure. The resulting gels have a distinctly different complement of proteins from that of the plasma membrane, suggesting a minimum of cross contamination between the two subcellular fractions. The adipocyte plasma membrane is composed of more than 20 polypeptides with a molecular weight range of 178,000–15,500 including two major glycoproteins of approximately 100,000 and 81,000 and several minor components. These molecular weight values are somewhat higher than those recently reported by Czech and Lynn (1973a). Furthermore, we observe at least three minor glycoprotein components whereas the above authors report the presence of only one minor component. It should be noted that the apparent molecular weights of the glycoproteins as analyzed on polyacrylamide gels may be subject to some ambiguity (Bretscher, 1971b; Segrest *et al.*, 1971). The electrophoretic patterns of the more than 20 resolved bands were completely reproducible in several gel runs, except for a variation in the position of the highest molecular weight band. The 178,000 band has been observed to run at a position corresponding to a molecular weight of approximately 310,000. The lower value is more consistent with the data of Czech and Lynn (1973a); however, other investigators have reported proteins with molecular weights greater than 300,000 in membranes from several other systems (Fairbanks *et al.*, 1971; Neville and Glossman, 1971). The possibility that the higher molecular weight band is converted under certain preparative conditions to the lower molecular weight component by enzymatic hydrolysis should be considered. However, under the conditions used for membrane isolation, no such variation occurs in the position of any other bands. The problem has not been resolved using this particular electrophoretic system.

The lactoperoxidase-catalyzed iodination technique has been used to label specifically the cell surface protein and perhaps glycoprotein components of intact adipocytes in order to determine the topology of the hormone-responsive plasma membrane. The absence of label in subcellular organelles suggests that those components labeled when intact cells were iodinated were located on the surface of the cell. Four distinct surface components occur in significant amounts in the plasma membrane. Two of these components (peaks 4 and 5) correspond in apparent molecular weight to the bands containing the major glycoprotein components. Czech and Lynn (1973b) have recently used the iodination technique to implicate two glycoproteins as surface components of adipocytes, as have Nachman *et al.* (1973) and Phillips and Morrison (1971a) investigating platelets and erythrocytes, respectively. When the iodination procedure was applied to the 24,000*g* pellet from homogenized adipocytes or to purified plasma membranes a considerably altered labeling pattern was obtained, suggesting that when the membrane was perturbed most of the proteins were now accessible to the reagent and could incorporate radioactive label.

To investigate whether the conditions used for iodination adversely affected the plasma membrane, the intact adipocytes were monitored for their ability to oxidize glucose to carbon dioxide. Neither the conditions used nor the actual covalent incorporation of iodine into the plasma membrane appeared to have any effect on the rate of CO_2 production as compared to untreated control cells. Because iodination did not interfere with the ability of the cell to perform a normal, complex metabolic function, the labeling pattern may be presumed to indicate which proteins are exposed on the surface of a functional adipocyte.

In all the iodination experiments a varying amount of radioactivity traveled ahead of the Bromophenol Blue marking dye on the polyacrylamide gels. Several studies on other membranes have indicated that a fast running component migrating at this position may be lipid or glycolipid (Salton and Schmitt, 1967; Lenard, 1970; Steck *et al.*, 1971; Salton *et al.*, 1972). When intact adipocytes were iodinated, the fast moving label accounted for a minor percentage of the

total incorporated activity; however, when the particulate fraction of the cell homogenate or the isolated plasma membrane was iodinated, this fast running band became a major radioactive component. When membranes were extracted prior to iodination, less than 5% of the radioactive label was found in this region as compared to the unextracted membrane. Vigorous extraction of iodinated membranes could remove all radioactivity from that position. These results suggest a marked difference in the exposure of this extractable membrane component between intact adipocytes and isolated plasma membranes. Based on the composition of adipocyte plasma membranes (Czech and Lynn, 1973a), this membrane component may possibly be lipid. Differences in lipid reactivity to acetic anhydride between whole red blood cells and intact ghosts have also been reported (Carraway *et al.*, 1972).

The labeling pattern of the homogenized and lipid extracted membrane systems suggests that all protein components in these preparations are available for reaction. However, the amount of radioactivity in peak 13 (Figures 4 and 5) is more than one would predict on the basis of protein concentration as assayed by Coomassie Blue stain. Czech and Lynn (1973b) also report unexpectedly high incorporation of iodine into a low molecular weight component. The Coomassie Blue stained gels of membrane preparations obtained before as well as after the iodination procedures had identical patterns. These observations preclude the possibility of protein hydrolysis in homogenized preparations resulting in a higher concentration of low molecular weight material which could subsequently be iodinated. It is suggested that the low molecular weight component may be particularly susceptible to iodination and that the intensity of staining by Coomassie Blue may not necessarily represent the true relative concentration of this component.

It was of interest to determine whether a change in the environment of the cell would cause a change in plasma membrane conformation detectable as an alteration in the proteins accessible to iodination. Different labeling patterns were obtained when the iodination of intact adipocytes was performed in Krebs-Ringer bicarbonate buffer or in phosphate-buffered saline containing EDTA, suggesting sensitivity to the incubation conditions. Insulin, in amounts that have profound effects on adipocyte metabolism (Rodbell, 1964), was shown to have an effect on plasma membrane protein accessibility to iodination which was, however, dependent on the medium in which the cells were suspended. Glucagon also appeared to cause an alteration in the distribution of iodine label bound by the protein components in the plasma membrane in either buffer system. Storm and Dolginow (1973) have suggested that glucagon may exert its hormonal effects on hepatocytes by inducing a conformational change in membrane components as measured by a differential inactivation of the adenylate cyclase system by iodoacetamide. A similar hormone-induced conformational change may be involved in adipocyte membranes.

Concanavalin A at concentrations of a new micrograms per milliliter has been shown to mimic the effects of insulin on adipocytes (Cuartrecasas and Tell, 1973). When this lectin is present at 100-fold higher concentrations, it appears to saturate binding sites on the cell surface (Cuatrecasas, 1973). Furthermore, it is thought to bind to carbohydrate moieties on mammalian cell surfaces (Sharon and Lis, 1972). Thus concanavalin A may be able to alter the accessibility of adipocyte surface glycoproteins to the lactoperoxidase-iodinating system. Changes similar to those effected by the addition of insulin were detectable using the iodination procedure when the lectin was present at low concentrations. However, a marked alteration in the labeling pattern was observed at a saturating concanavalin A concentration, suggesting that the lectin may be partially masking a glycoprotein from the iodinating system and/or inducing conformation changes in the plasma membrane which result in an altered susceptibility to iodination.

The results of these studies suggest that several components of the adipocyte plasma membrane are situated on the outer surface of this hormone-responsive membrane. The lactoperoxidase-catalyzed iodination technique has also been successfully used to detect alterations in the exposure of protein components in the adipocyte plasma membrane caused by substances which have been shown to regulate adipocyte metabolism *in vivo* and *in vitro*. These changes in the plasma membrane may be involved in the mechanism of hormone regulation. A characterization of hormone receptors and a description of the effects of hormones on membrane structure and function should contribute to a greater understanding of the mechanism of hormone action.

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References

Avruch, J., Carter, J. R., and Martin, D. B. (1972), *in* Handbook of Physiology, Section 7: Endocrinology, Vol. 1, Steiner, D. F., and Freinkel, N., Ed., Baltimore, Md., Williams and Wilkins, p 545.

Berg, H. C. (1969), *Biochim. Biophys. Acta 183,* 65.

Birnbaumer, L., Pohl, S. L., and Rodbell, M. (1969), *J. Biol. Chem. 244,* 3468.

Birnbaumer, L., and Rodbell, M. (1969), *J. Biol. Chem. 244,* 3477.

Bligh, E. G., and Dyer, W. J. (1959), *Can. J. Biochem. Physiol. 37,* 911.

Bretscher, M. S. (1971a), *J. Mol. Biol. 59,* 351.

Bretscher, M. S. (1971b), *Nature (London), New Biol. 231,* 229.

Carraway, K. L., Kobylka, D., Summers, J., and Carraway, C. A. (1972), *Chem. Phys, Lipids 8,* 65.

Carter, J. R., Avruch, J., and Martin, D. B. (1972), *J. Biol. Chem. 247,* 2682.

Cuatrecasas, P. (1969), *Proc. Nat. Acad. Sci. U. S. 63,* 450.

Cuatrecasas, P. (1972), *J. Biol. Chem. 247,* 1980.

Cuatrecasas, P. (1973), *Biochemistry 12,* 1312.

Cuatrecasas, P., and Tell, G. P. E. (1973), *Proc. Nat. Acad. Sci. U. S. 70,* 485.

Cunningham, B. A., Wang, J. L., Pflumm, M. N., and Edelman, G. M. (1972), *Biochemistry 11,* 3233.

Czech, M. P., and Lynn, W. S. (1973a), *J. Biol. Chem. 248,* 5081.

Czech, M. P., and Lynn, W. S. (1973b), *Biochemistry 12,* 3597.

Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971), *Biochemistry 10,* 2606.

Gurd, J. W., Evans, W. H., and Perkins, H. R. (1972), *Biochem. J. 126,* 459.

Hartree, E. F. (1972), *Anal. Biochem. 48,* 422.

Hubbard, A. L., and Cohn, Z. A. (1972), *J. Cell. Biol. 55,* 390.

Huber, C. T., and Morrison, M. (1973), *Biochemistry 12,*

4274.
Jeanrenaud, B., and Hepp, D., Ed. (1970), *Horm. Metab. Res., Suppl. 2: Adipose Tissue: Regul. Metab. Funct.*
Kreibich, G., Hubbard, A. L., and Sabatini, D. D. (1974), *J. Cell. Biol 60,* 616.
Laudat, M. H., Pairault, J., Bayer, P., Martin, M., and Laudat, Ph. (1972), *Biochim. Biophys. Acta 255,* 1005.
Lenard, J. (1970), *Biochemistry 9,* 1129.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265.
Marchalonis, J. J. (1969), *Biochem. J. 113,* 299.
Marchalonis, J. J., Cone, R. E., and Santer, V. (1971), *Biochem. J. 124,* 921.
McKeel, D. W., and Jarett, L. (1970), *J. Cell. Biol. 44,* 417.
Mehrishi, J. N. (1972), *Progr. Biophys. Mol. Biol. 25,* 1.
Nachman, R. L., Hubbard, A., and Ferris, B. (1973), *J. Biol. Chem. 248,* 2928.
Neville, D. M., Jr., and Glossman, H. (1971), *J. Biol. Chem. 246,* 6335.
Phillips, D. R. (1972), *Biochemistry 11,* 4582.
Phillips, D. R., and Morrison, M. (1970), *Biochem. Biophys. Res. Commun. 40,* 284.
Phillips, D. R., and Morrison, M. (1971a), *Biochemistry 10,* 1766.
Phillips, D. R., and Morrison, M. (1971b), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 18,* 95.
Poduslo, J. F., Greenberg, C. S., and Glick, M. C. (1972), *Biochemistry 11,* 2616.
Rifkin, D. B., Compans, R. W., and Reich, E. (1972), *J. Biol. Chem. 247,* 6432.
Rodbell, M. (1964), *J. Biol. Chem. 239,* 375.
Rodbell, M. (1966), *J. Biol. Chem. 241,* 140.
Salton, M. R. J., and Schmitt, M. D. (1967), *Biochem. Biophys. Res. Commun. 27,* 529.
Salton, M. R. J., Schor, M. T., and Ng, M. H. (1972), *Biochim. Biophys. Acta 290,* 408.
Segrest, J. P., Jackson, R. L., Andrews, E. P., and Marchesi, V. T. (1971), *Biochem. Biophys. Res. Commun. 44,* 390.
Sharon, N., and Lis, H. (1972), *Science 177,* 949.
Steck, T. L., Fairbanks, G., and Wallach, D. F. H. (1971), *Biochemistry 10,* 2617.
Storm, D. R., and Dolginow, Y. D. (1973), *J. Biol. Chem. 14,* 5208.

Effect of *N*-Ethylmaleimide and of Heat Treatment on the Binding of Dynein to Ethylenediaminetetraacetic Acid Extracted Axonemes†

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ABSTRACT: Cilia from *Tetrahymena pyriformis* were extracted with Tris-EDTA to yield crude dynein which for some experiments was further purified by centrifugation on sucrose density gradients to yield 30S dynein. Dynein was incubated with EDTA extracted axonemes for 30 min at 25° and the axonemes precipitated by centrifugation. The amount of dynein bound to the axonemes was computed from the loss of ATPase activity in the supernate and from the gain of ATPase activity in the pellet. In three-fourths of the experiments more activity appeared in the pellet than disappeared from the supernatant, indicating an enhancement of the ATPase activity of dynein upon binding to EDTA extracted axonemes. If dynein were reacted with *N*-ethylmaleimide (NEM) or heated at about 40–46° before incubation with native EDTA extracted axonemes, the dynein ATPase activity increased but its ability to bind to the axonemes and to display an enhancement of its ATPase activity on binding were reduced. If EDTA extracted axonemes were reacted with NEM prior to incubation with native dynein, there was little effect on their ability to bind the dynein, but if the axonemes were heated prior to the incubation with native dynein, the ability of the axonemes to bind the native dynein and to enhance the ATPase activity of the bound dynein was reduced. Thus an SH group(s) on the dynein but not on the EDTA extracted axonemes is required for binding of the dynein to the axonemes and for enhancement of the ATPase activity upon binding, whereas both sets of binding sites are very sensitive to thermal denaturation.

It is now established (Summers and Gibbons, 1971) that the ability of cilia and flagella to generate bending waves is based upon a sliding filament system. The paired arms projecting from the A subfibers of each outer doublet are thought to act as cross bridges that contact the B subfiber of the adjacent doublet and generate the sliding motion which, in the presence of shear resistant linkages (Brokaw, 1966; Lubliner and Blum, 1971), will lead to bending. Gibbons (1965a) showed that extraction of *Tetrahymena* cilia by Tris-EDTA led to the appearance of two forms of the ATPase dynein in solution and the disappearance of the arms. His finding that the 30S dynein could be rebound to the extracted axonemes with the reappearance of the arms and that ATP caused a light-scattering change of the axonemes only if 30S dynein had been added indicated that the 30S form was the mechanochemically effective ATPase of cilia and that it was a major component of the arms (Gibbons, 1965b). Since the arms are not the same shape other

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